41. Thickness measurement of Actin-Bundle in solution(poster presentation,Soft Matter as Structured Materials)

AUTHOR(S):

Watanabe, Shun

CITATION:

Watanabe, Shun. 41. Thickness measurement of Actin-Bundle in solution(poster presentation,Soft Matter as Structured Materials). 物性研究 2005, 84(6): 942-943

ISSUE DATE:

2005-09-20

URL:

http://hdl.handle.net/2433/110288

Thickness measurement of Actin-Bundle in solution

Dept. of Physics, Kyoto Univ. Shun Watanabe [1]

剛直性繊維のタンパク質アクチンフィラメントは多価のカチオンを添加するとバンドル状に凝集することは報告されたが、その凝集は光散乱強度で見ると急激な変化をしているが連続的になっている。その中間域でのアクチンフィラメント溶液を蛍光顕微鏡で観察し、その蛍光強度密度によりその凝集状態を調べたところ、中間域では単独のフィラメントと多数本凝集したバンドルが共存している事がわかった。

Actin-filaments are very stiff fibers constructed by protein actin. Oriol-Audit et al,[1] and J.X. Tang et al.[2] reported that not protein molecule, spermidine, cationic polyaminoacid, caused bundle formation *in-vitro*. Actin-filament has large negative charge wholly. Multivalent cations may work as paste, then actin-filaments condense and form bundles.

In generary, these research detected occurrence of bundle formation by significant increase of light scattering intencity of solution. Fig. 1 shows the typical characterisitic scattaring curve. At a concentration, light scattering intencity significantly increases and until it get to upper limit, continuously. We called this range middle range. Previous researchs didn't pay attention to the middle range. But it is important for thermodynamics that which structure actin-filaments form in the middle range solution.

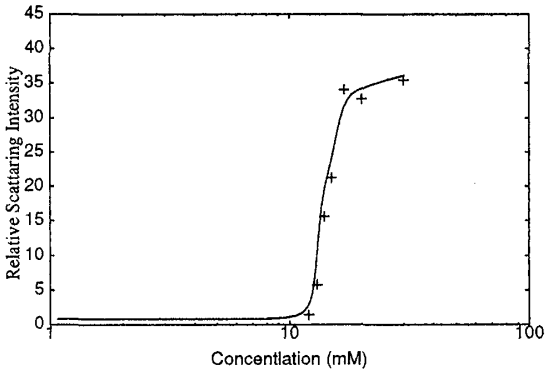

Figure 1: scattaring intencity of solution added Spermidine(+3)
This is light scattaring intensity of various concentration spermidine(+3) solutions. Intensity is relative value to no spermidine solution. Light scattaring curve significantly but continuous increase with spermidine concentration and get to upper limit around 12 mM. It was measured with a luminescence spectrometer at light wave length 450 nm.

We observe the actin-filament solutions on 'the middle range' by using a fluorescent microscope. Fig. 2 is one of our results. Actin-filaments thickness is smaller than the resolution of

[1]E-mail: watanabe@chem.scphys.kyoto-u.ac.jp

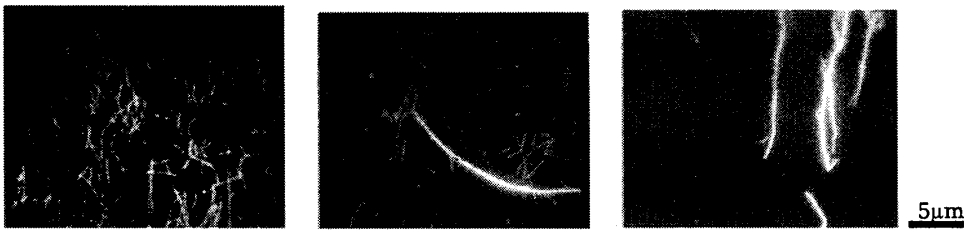

Figure 2: Fluorescent microscope images of actin-filament solution added spermidine(+3) These images are actin filament in various concentration spermidine(+3) solutions.

visible light microscope. But fluorescent intensity is proportional to density of actin filaments, therefore we can measure relative thickness of the bundle in solution by fluorescent microscope. Week fluorescent line images on the leftest picture are single actin-filaments, which have almost same intensity. Strong fluorescent line images on the rightest picture are bundles of actin-filaments. These have about 6 times of single filament's intensity. What the most interesting results is the center picture. Week fluorescent images and Stronge ones are coexist on the center picture which is in 'the middle range' solution.

From these results, we can conclude about the mechanism of bundle transformation as the following. In lower concentration of multivalent cation solution, the bundle form is unstable. At the state of 'the middle range', both single actin-filaments and the bundles which contains certain filaments are stable. Then at sufficient hight concentration solution, no longer single actin-filaments are stable, but the bundles are stable. This type mechanism should be a kind of first order phase transition, limited in a small systems[3].

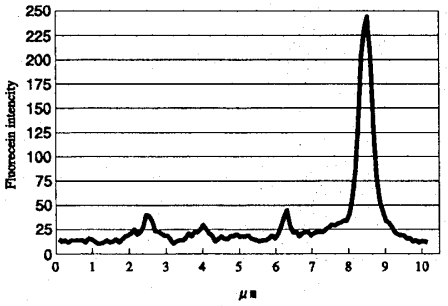

Figure 3: Intensity on the dotted-line of middle image of figure 2

3 small peaks are almost same intensity. They correspond to single filament. The big peak corresponds to bundle of actin-filament.

References

[1] C. Oriol-Audit. *Biochem. Biophys. Res. Comm.*, **105** (1982), 1096.

[2] J. X. Tang, et. al. *Ber. Bundrnges. Phys. Chem.*, **100** (1996), 796.

[3] K. Yoshikawa, et. al. *Phys. Rev. Lett.*, **76** (1996), 3029.